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REVIEW

Hypoxic-Ischemic Encephalopathy: Pathogenesis and Promising Therapies

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Abstract

Hypoxic-ischemic encephalopathy (HIE) is a brain lesion caused by inadequate blood supply and oxygen deprivation, often occurring in neonates. It has emerged as a grave complication of neonatal asphyxia, leading to chronic neurological damage. Nevertheless, the precise pathophysiological mechanisms underlying HIE are not entirely understood. This paper aims to comprehensively elucidate the contributions of hypoxia–ischemia, reperfusion injury, inflammation, oxidative stress, mitochondrial dysfunction, excitotoxicity, ferroptosis, endoplasmic reticulum stress, and apoptosis to the onset and progression of HIE. Currently, hypothermia therapy stands as the sole standard treatment for neonatal HIE, albeit providing only partial neuroprotection. Drug therapy and stem cell therapy have been explored in the treatment of HIE, exhibiting certain neuroprotective effects. Employing drug therapy or stem cell therapy as adjunctive treatments to hypothermia therapy holds great significance. This article presents a systematic review of the pathogenesis and treatment strategies of HIE, with the goal of enhancing the effect of treatment and improving the quality of life for HIE patients.

Keywords HIE · Pathogenesis · Hypothermia therapy · Drug therapy · Stem cell therapy

Introduction

Hypoxic-ischemic encephalopathy (HIE) is a form of brain injury resulting from hypoxia and reduced cerebral blood flow, leading to mortality and long-term neurological sequelae [1, 2]. Perinatal asphyxia can result in fetal or neonatal brain damage with the pathology evolving over time [3]. Neonatal HIE represents a subset of neonatal encephalopathy stemming from perinatal asphyxia and constitutes a significant condition associated with acute neonatal mortality and chronic neurological impairment. The incidence of neonatal HIE is 2–3 out of every 1000 newborns in developed countries and 26 out of every 1000 newborns in developing countries, with approximately 10–15% of asphyxiated newborns with HIE dying during the neonatal period, and another 25–30% experiencing long-term sequelae [4–6]. Neonatal HIE is not only a great harm to patients themselves, but also a serious harm to families and society. Collaborative research efforts all over the world are essential to reduce its incidence and enhance treatment efficacy.

The occurrence of HIE is a continuous process that leads to neuronal cell death within hours after the initial injury. The pathogenesis of HIE can be mainly summarized as the following process (Fig. 1): Firstly, it is the primary phase, during which ischemia and hypoxia cause primary energy failure, leading to oxidative metabolic failure, accumulation of excitotoxin, cell death, intracellular Ca^{2+} accumulation, and edema. Secondly, it is the latent period, during which the organism restores respiration and cerebral circulation, leading to reperfusion injury and oxidative stress [7, 8]. At the same time, accompanied by the emergence of neuroinflammation and mitochondrial dysfunction, along with the activation of pro apoptotic signaling pathways, secondary

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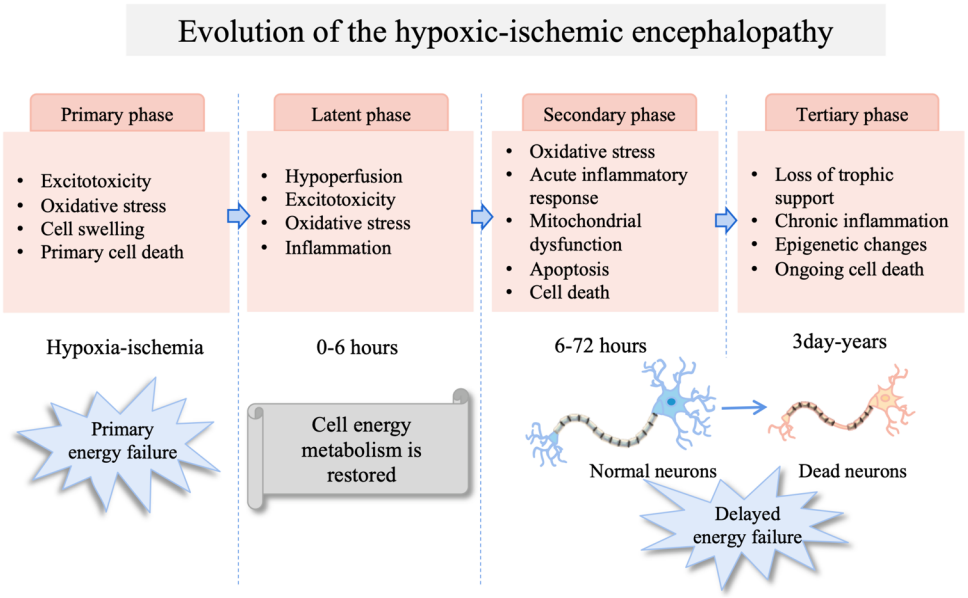

Fig. 1 Evolution of the HIE

energy failure (delayed neuronal death) is induced, causing adverse reactions to the body such as secondary cell death, excitotoxicity, and delayed maturation of oligodendrocytes. With continuous inflammation and epigenetic changes, brain damage can persist for weeks, months, or even years, ultimately causing serious damage to the body [1, 9]. The pathogenesis of HIE mainly includes hypoxia–ischemia, reperfusion injury, inflammation, oxidative stress, mitochondrial dysfunction, excitotoxicity, ferroptosis, endoplasmic reticulum stress (ERS), cell swelling, impaired maturation, and apoptosis [10]. However, currently, the only clear and effective treatment method in clinical practice is therapeutic hypothermia. The mortality rate caused by HIE are still high, and the research on HIE is very important and meaningful.

The Pathogenesis of HIE

The progress of HIE can be divided into four phases, although it is actually a continuous process with potential interference among phases [8, 11]. The primary phase is characterized by primary energy failure during hypoxic-ischemic events, leading to harmful reactions such as ATP-dependent pump blockage, lactic acidosis, intracellular accumulation of calcium ions, excitatory amino acids release, toxic edema formation, and necrosis in the most sensitive areas of the brain [1]. Prior to the secondary phase, there is an incubation period, the energy recovery phase of resuscitation [12]. However, after 6–72 h (Phase 2), the brain's energy depletion process reoccurs in areas with greater resistance, maintenance of excitotoxicity, large influx of ionic calcium, increased activation of neuronal NO synthase, oxidative stress and mitochondrial dysfunction will lead to secondary energy failure and programmed neuronal death through activation of caspase pathway. It is characterized by the deterioration of mitochondrial function and acute inflammatory response, leading to apoptosis at this phase [13]. The tertiary phase involves sustained inflammatory response and epigenetic changes. In this pathological process, oxidative stress can lead to direct central nervous system damage and activate a cascade of inflammatory responses that promote the development of the tertiary phase; persistent inflammation aggravates the damage [1, 14].

The Primary Phase

Ischemia and Hypoxia

The initial phase of HIE mainly manifests as cerebral tissue ischemia and hypoxia. Hypoxic-ischemic brain injury deprives patients of oxygen and glucose in the brain. The decrease in oxygen inhibits the normal function of the tricarboxylic acid cycle and electron transfer chain, inhibits oxidative phosphorylation, and leads to rapid depletion of ATP, resulting in ATP deficiency. The activity of ATP dependent ion pumps decreases, and the failure of cell membrane ion pumps leads to Na^+ and Ca^{2+} accompanying water flow into cells, leading to intracellular Ca^{2+} accumulation, edema, and cell death [15, 16]. In addition, hypoxia can be supplemented through anaerobic metabolism, which can lead to lactic acidosis [1]. Although there is usually only limited cell death during ischemia and hypoxia, the indirect damage it causes to the body through influencing other factors is very serious [17]. Therefore, intervention and treatment are important at this phase.

Excitotoxicity

Excitotoxicity is believed to be a common pathway for a large number of neurological diseases, mainly referring to the toxic effects of the excitatory neurotransmitter glutamate. Long-term activation of the glutamate receptor can trigger neurotoxicity, ultimately leading to loss of neuronal function and cell death [18]. Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS) of mammals, and N-methyl-D-aspartate receptor (NMDAR) is a receptor for glutamate [19]. After cerebral ischemia and hypoxia, energy consumption and ion imbalance are induced, and the lack of ATP hinders the physiological function of ion pumps. Na^+ and Ca^{2+} abnormally flow into cells, accompanied by K^+ efflux, leading to a significant increase in extracellular K^+ concentration and neuronal depolarization, further leading to the inability of Ca^{2+} to be pumped out of cells. High Ca^{2+} levels in turn trigger a large release of glutamate in the synaptic gap [20]. High levels of glutamate in synaptic gap bind to NMDAR, and excessive excitation of NMDAR leads to additional Ca^{2+} entering the cells, which causes the cells to become overexcited. Overexcitation of cells leads to the release of toxic substances, further promoting cell death and forming a vicious cycle (Fig. 2). In addition, excessive stimulation of other glutamate receptor families can also trigger the influx of Na^+ and Cl^-, accompanied by the diffusion of water to counteract osmotic pressure, leading to cell swelling [18]. Excessive Ca^{2+} intake triggers molecular neurotoxic cascades, further leading to excessive production of free radicals, mitochondrial dysfunctions, release of apoptotic substances, cell membrane damage, and DNA breakage, all of which ultimately lead to cell apoptosis or necrosis [1, 20]. In addition, the most common free radicals induced by excitotoxicity are reactive

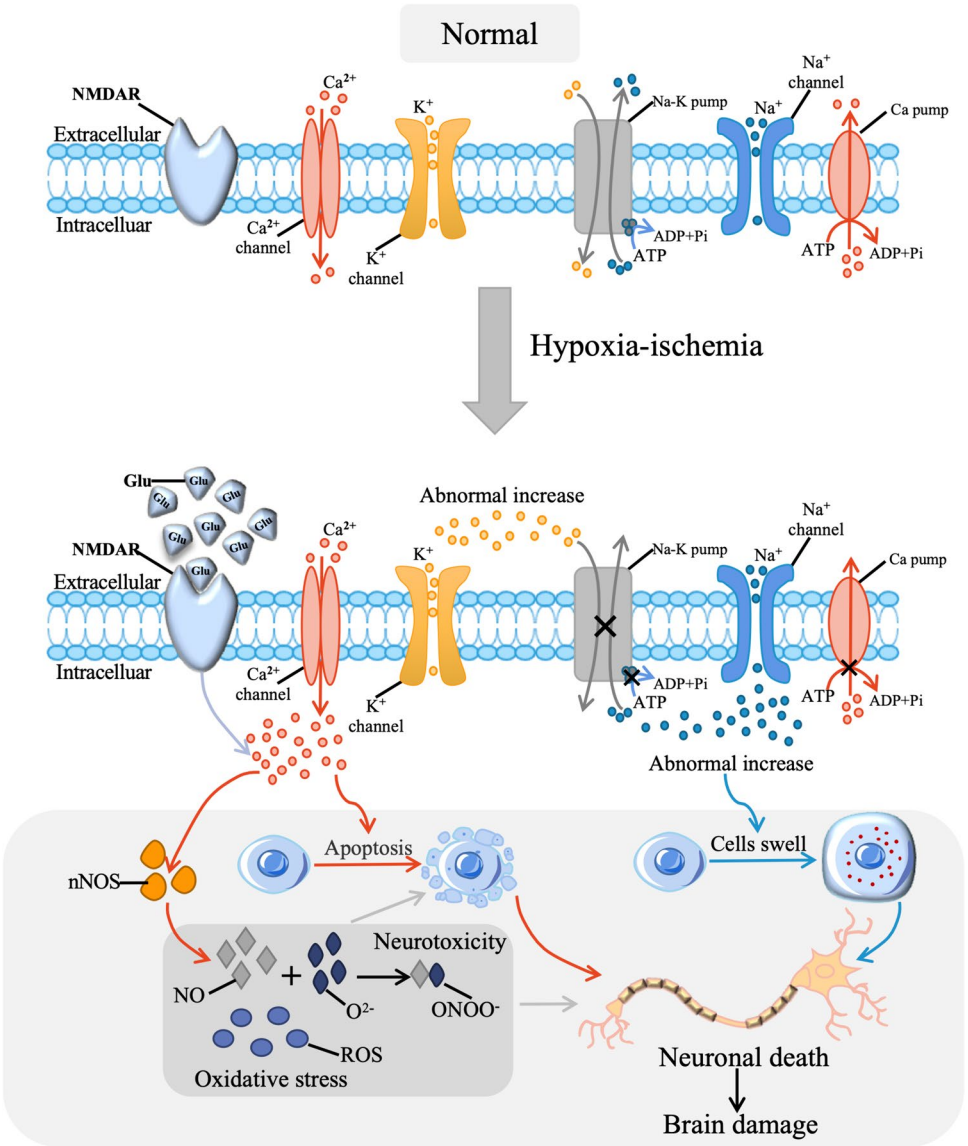

Fig. 2 Excitotoxicity and oxidative stress in the process of HIE

oxygen species (ROS) and reactive int rogen species (RNS), which are derived from oxygen and nitric oxide (NO). Neuronal nitric oxide synthase (nNOS) is overactivated by a large influx of Ca^{2+}, producing excessive NO, which reacts with superoxide ion (O2(-)) to produce toxic peroxynitrite (ONOO(-)), leading to neuronal toxicity and cell death[19, 20]. NO can also inhibit the activity of cytochrome oxidase, thereby affecting mitochondrial respiratory function. Inhibiting NOS activity can reduce iron deposition and NO generation, thereby reducing neuronal death. In short, excitotoxicity can exacerbate neuronal loss of function and death by affecting a variety of factors, therefore, excitatory amino acid inhibitors or glutamate receptor antagonists are good target of future research to reduce brain damage.

The Secondary Phase

Latent Period: Ischemia–Reperfusion (I/R)

There is a latent period of about 6 h after hypoxia and ischemia, with recovery of blood flow and oxygen supply, a brief recovery of oxidative metabolism, and even partial or complete recovery of many brain cells after reperfusion [11, 17, 21]. However, restoring respiration and circulation will lead to reperfusion injury and oxidative stress. During this process, necrotic cells die within minutes to hours after returning to normal oxygenation and sufficient perfusion [13], which causes more serious damage. Although there was a brief recovery period earlier, during the reperfusion process, primary energy failure could not be compensated, leading to secondary energy failure [22]. In the secondary phase (6–72 h), the excitotoxicity state is maintained, mitochondrial function deteriorates, oxidative stress is secondary to membrane potential changes, and ATP synthesis decreases in the intracellular alkaline toxic environment [1]. Next, we will mainly elaborate on the mechanisms involved in the secondary phase of HIE, including oxidative stress, acute inflammatory response, mitochondrial dysfunction, and apoptosis.

Oxidative Stress and Acute Inflammatory Response

Excitotoxicity occurs in the primary phase, which further promotes Ca^{2+} influx into cells, and the further influx of calcium also leads to increased lipase activation, leading to fatty acid release, increased nNOS activation, free radical production, and mitochondrial dysfunction [15, 23]. ROS is mainly produced in mitochondria. Under normal circumstances, ROS is usually cleared by superoxide dismutase (SOD) and glutathione peroxide (GPX). During hypoxia, due to metabolic disruption, ROS cannot be immediately cleared by antioxidant enzymes, resulting in excessive accumulation of ROS [24]. Reperfusion contributes to further damage caused by the excessive production of free radicals, leading to secondary ATP loss and subsequent brain cell apoptosis. In addition, oxygen deprivation, energy consumption, and reoxidation lead to the production of ROS, causing oxidative damage to membranes, proteins, lipids, and DNA, thereby interfering with their functions [14, 15]. ROS leads to rapid activation of astrocytes and microglia, which secreting pro-inflammatory cytokines (IL-6, TNF-α, IL-1α, and IL-1β) and IFN-γ, leading to inflammatory response [14]. These derivatives participate in complex interactions across multiple pathways such as inflammation, apoptosis, autophagy, and necrosis, ultimately leading to brain damage.

Mitochondrial Dysfunction and Apoptosis

Perinatal asphyxia causes ischemia and hypoxia, depleting the energy of tissues that rely solely on aerobic metabolism, such as the CNS [25]. Research has shown that mitochondrial dysfunction is the key to neurodegeneration in HIE [26]. Mitochondria also produce ROS during the process of producing energy required by cells. However, during hypoxia and ischemia, metabolism is disrupted, and ROS cannot be immediately cleared by antioxidant enzymes (Fig. 3). Therefore, excessive accumulation of ROS occurs, which, together with oxidative modification of lipids, proteins, and DNA, leads to metabolic failure and mitochondrial dysfunction in newborns after HI brain injury [24]. Mitochondrial dysfunction can also lead to the accumulation of Ca^{2+} in cells, leading to neurotoxicity [19]. In summary, the series of problems caused by mitochondrial dysfunction, such as oxidative stress, intracellular Ca^{2+} accumulation, and mitochondrial energy failure, work together to lead to neurodegeneration.

In addition, mitochondrial dysfunction also triggers apoptosis (Fig. 3). Mitochondrial dysfunction leads to excessive accumulation of ROS, which can trigger the formation of mitochondrial permeability transition pore (mPTP) [24, 27, 28]. The mPTP allows proapoptotic factors such as apoptotic protease activating factor-1 (Apaf-1) and cytochrome c to be released from mitochondria into the cytoplasm. Cytochrome c interacts with Apaf-1 to form apoptotic bodies, activating cysteinyl aspartate specific proteinase-9 (caspase-9) in the cytoplasm and initiating an apoptotic cascade reaction, ultimately leading to apoptotic cell death [29, 30]. In adult animal models, mPTP inhibition has neuroprotective effects [31]. Studies have shown that neonatal HI brain injury is associated with the translocation of proapoptotic protein B-cell lymphoma-2 associated X protein (Bax) to

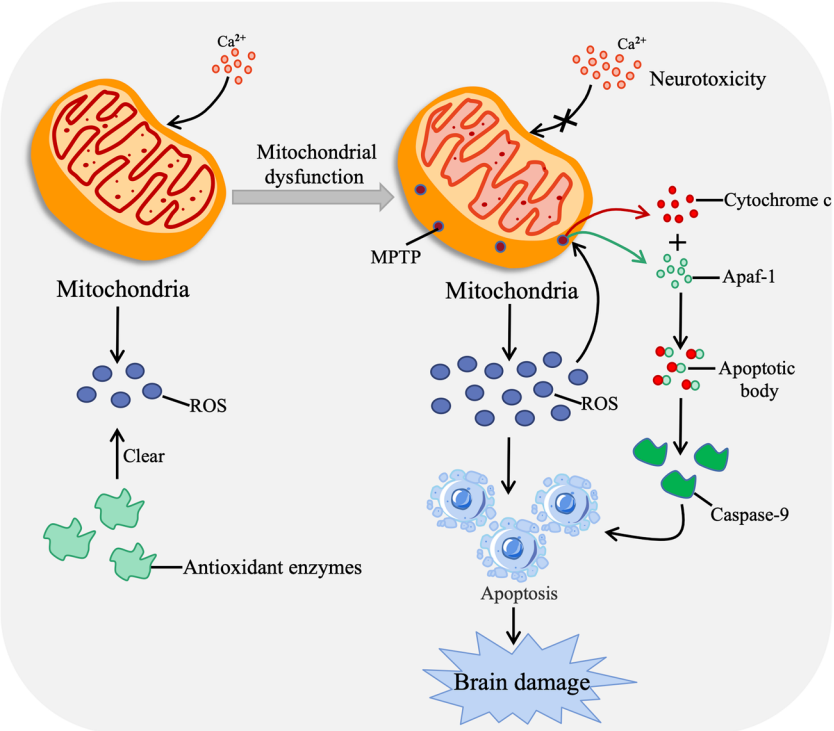

Fig. 3 Mitochondrial dysfunction and apoptosis in the process of HIE

mitochondria, while a decrease in B-cell lymphoma-2 (Bcl-2) leads to activation of caspase-3, leading to apoptosis, which reaches its peak 24 to 72 h after HI brain injury [2, 32]. This indicates that apoptosis plays a driving role in the pathological development of HIE, but the specific mechanism still needs further research.

The Tertiary Phase

Persistent Inflammation and Epigenetic Changes

Inflammation plays a crucial role in HIE and can mediate secondary neuronal death (Fig. 4). The excessive activation of inflammatory response in HIE is closely related to microglia, astrocytes, and neutrophils [33]. During ischemia, neutrophils immediately infiltrate the damaged brain tissue, exacerbating brain damage through various mechanisms, including the production of ROS, the release of pro-inflammatory cytokines, the reduced microvascular flow due to neutrophil blockage of capillaries, the increased matrix metalloproteinase-9 (MMP-9) secretion, ultimately leading to the breakdown of blood brain barrier (BBB) [34–36]. Microglia is key mediator of inflammation during perinatal brain injury, and its activation and aggregation have been recognized as pathological markers of HIE in infants [33, 37]. Microglia play both pro-inflammatory (type M1) and anti-inflammatory (type M2) roles in neuronal damage and recovery due to their two phenotypes[38, 39]. When ischemic events occur, microglia are rapidly activated and show macrophage like abilities, including phagocytosis and the production of pro-inflammatory cytokines (IL-1 and TNF-α) and chemokines (MCP-1 and MIP-1α). Among them, pro-inflammatory cytokines and chemokines can exert neurotoxic functions by recruiting leukocyte from the periphery. The recruited neutrophils, lymphocytes, and natural killer cells release free ROS and proteases, increasing the permeability of BBB, leading to the destruction of BBB and the swelling and degeneration of neurons and glial

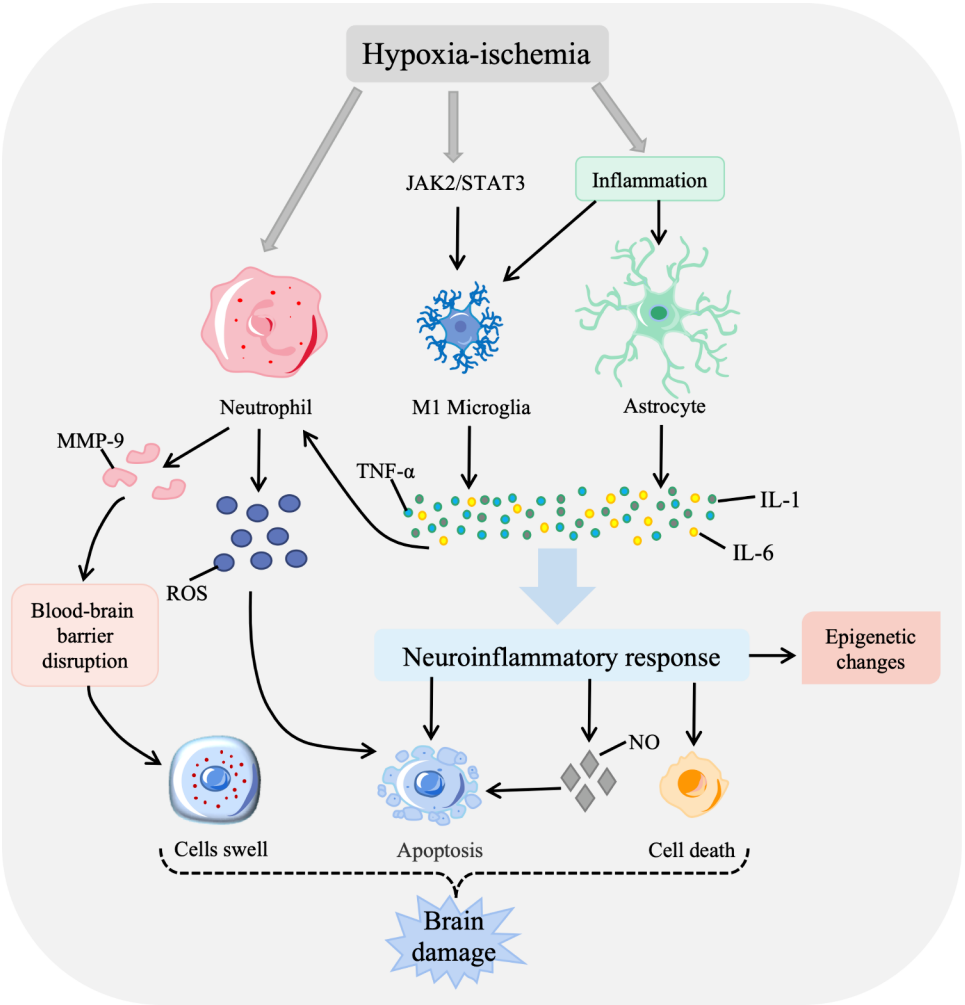

Fig. 4 Chronic inflammation in the process of HIE

cells, thereby causing damage to ischemic tissue [36, 40, 41]. Studies have shown that hypoxia ischemia may activate the janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signaling pathway, leading to microglial activation and neuroinflammation. Downregulation of the JAK2/STAT3 signaling pathway can inhibit the activation of microglia, thereby inhibiting or alleviating inflammatory damage to the nervous system [42, 43]. Our recent finding in rat pups indicated that interleukin-35 (IL-35) effectively inhibits the polarization and activation of microglia, reduces damage to vascular endothelial cells, and thus alleviates HIE brain damage [44]. Importantly, we have found that IL-35's protective effects are closely associated with the regulation of hypoxia-inducible factor-1α (HIF-1α) and nuclear factor-κB (NF-κB) signaling pathways [44–46].

In addition to microglia, astrocytes are activated due to inflammatory response, and overactivated astrocytes secrete a large amount of inflammatory factors, such as IL-6, TNF-α, IL-1, and interferon-gamma, inducing cascade amplification of neuroinflammatory response, leading to neuronal apoptosis, inhibiting neurogenesis, and exacerbating ischemic damage [33, 47, 48]. Furthermore, reactive astrocytes also secrete chemokines after ischemia, causing immune cells to be attracted to the ischemic site, exacerbating brain damage. Research has shown that astrocytes have the ability to upregulate the expression of inflammatory mediators in the inflammatory response induced by neonatal HIE [33]. Additionally, the increase in hydrogen peroxide (H_2O_2) and NO levels, as well as the excessive production of free radicals and ROS, together with persistent inflammation, stimulates necrosis and apoptosis [22, 49, 50].

Persistent inflammation is also accompanied by epigenetic changes, which can lead to delayed maturation of oligodendrocytes, and impaired neurogenesis, axonal growth and synapse, thereby causing further damage to HIE patients [51]. It is worth noting that the tertiary phase can last for several weeks to years, and this prolonged injury may be exacerbated by persistent inflammation [21, 52]. Inflammation is closely related to exacerbating brain injury post ischemia, and further research on the inflammatory mechanism in HIE, as well as studying the regulatory mechanisms

of microglia, astrocytes, and neutrophils, holds promise for improving treatment efficacy and patient prognosis.

Other Mechanisms Involved in HIE

Ferroptosis

The intracellular iron exists in the form of divalent iron ions (Fe^{2+}) and trivalent iron ions (Fe^{3+}). Iron plays a crucial role as a cofactor in the metabolism of enzymes and catalysts, involved in various redox processes. Under the condition of accumulation of Fe^{2+}, lipid peroxide can be reduced by Fe^{2+} to generate highly reactive alkoxy groups. These free radicals can directly destroy adjacent polyunsaturated fatty acid, causing a large amount of lipid related ROS accumulation, leading to the intracellular ferroptosis [53]. Ferroptosis, as a programmed cell death, is different from the traditional programmed cell death. It is mainly manifested as cell death caused by the over-accumulation of iron dependent lipid peroxide, related to glutamite (GSH) metabolism, lipid peroxidation, and abnormal iron metabolism, and is involved in the occurrence and development of many diseases [54, 55]. Glutathione peroxidase 4 (GPX4) and GSH are essential proteins for ferroptosis. GSH is an intracellular Fe^{2+} ligand, and is essential for GPX4 activity. Decreased GSH levels lead to the production of toxic hydroxyl radicals and inactivation of GPX4 causes excessive accumulation of peroxidized lipids, resulting in cellular ferroptosis [56, 57]. Asphyxia could affect iron metabolism in newborns with HIE, leading to a significant increase in non-protein bound iron (NPBI) [58]. Recently, studies have reported that neonatal hypoxia and ischemia lead to a decrease in the expression of GPX4 and GSH in brain tissue, a decrease in antioxidant capacity, an increase in ROS expression, and a significant increase in lipid peroxidation levels. The acidic environment caused by ischemia and hypoxia may lead to the accumulation of free Fe^{2+} in brain tissue, which is consistent with the development of ferroptosis [56]. Ferroptosis may be a developing potential treatment in the pathogenesis of neonatal HIE. Although ferroptosis is known to play a role in the pathological process of HIE, little is known about its endogenous mechanisms. Currently, targeting the GPX4 pathway has become a starting point for the treatment of HIE.

Endoplasmic Reticulum Stress

Low energy status has detrimental effects on protein homeostasis, impacting protein folding, maturation, and degradation regulated by the ubiquitin proteasome and autophagy functions. Consequently, endoplasmic reticulum (ER) function is impaired, leading to endoplasmic reticulum stress (ERS) [30, 59, 60]. The occurrence of oxidative stress and calcium overload after cerebral ischemia–reperfusion further exacerbates the accumulation of misfolded proteins and ERS [61]. In the early phases of brain tissue ischemia–reperfusion, neural cells activate various survival mechanisms to mitigate stress responses and restore stability in the endoplasmic reticulum environment. Our previous various researches have demonstrated that moderately activated ERS and unfolded protein response (UPR) have a certain protective effect against cerebral ischemic injury [62–64]. However, in cases of persistent and severe ERS caused by cerebral ischemia, UPR is unable to restore damaged endoplasmic reticulum function, leading to related apoptotic pathways. Study has found that after HIE, the expression of glucose regulated protein 78 (GRP78), heat shock protein 70 (Hsp70), C/EBP homologous protein (CHOP), and phosphorylated of eukaryotic initiation factor-2α (eIF2α) significantly increases, indicating the involvement of ERS in brain damage induced by HIE [65]. CHOP, as a pro-apoptotic factor, can decrease the expression of the anti-apoptotic protein Bcl-2, leading to increased permeability of the mitochondrial membrane and initiation of the mitochondrial apoptosis pathway [30]. Persistent or excessive ERS can also result in cysteine aspartic acid specific protease-12 (caspase-12) enzyme entering the cytoplasm from the endoplasmic reticulum, subsequently activating caspase-9 and caspase-3, and ultimately leading to nuclear DNA division and triggering endoplasmic reticulum stress-mediated cell apoptosis [66]. Neonatal hypoxic-ischemic injury can lead to endoplasmic reticulum stress, which exacerbates oxidative stress and disrupts cellular balance, ultimately impacting long-term neurological function [67]. The regulation of ERS could provide a therapeutic approach to obstruct the pathological processes caused by HIE.

Treatment Strategies for HIE

A number of potential neuroprotective strategies targeting different pathways have been investigated in several clinical trials and various animal models of neonatal HIE. In this review, the therapeutic studies on HIE are summarized from three aspects: standard therapy, drug therapy (Table 1), and stem cell therapy.

Standard Treatment Method: Therapeutic Hypothermia

Therapeutic hypothermia is the only standard treatment method today and is widely used for neonatal HIE [8, 10]. Therapeutic hypothermia is the cooling of the body or brain and is the most powerful neuroprotective methods at preclinical and clinical levels. The main protective mechanism of therapeutic hypothermia is that the brain metabolic rate decreases with the decrease of temperature. In addition, the

Table 1 Molecular mechanisms of neuroprotective drugs

Drugs/effect	Anti-inflammatory	Anti-apoptotic	Anti-excytotoxic	Antioxidant	Neurotrophic	References
Allopurinol				✓		[24, 68–70]
Melatonin	✓	✓	✓	✓		[11, 71–75]
Erythropoietin	✓	✓	✓	✓	✓	[76–82]
Topiramate	✓	✓	✓			[83–88]
Cerebrolysin	✓	✓	✓	✓	✓	[89, 90]
Magnesium sulfate			✓			[91]
N-acetyl-l-cysteine	✓	✓		✓		[92–94]
Hydrogen,	✓	✓		✓	✓	[95, 96]
Xenon	✓	✓	✓	✓		[97, 98]
Cannabidiol	✓	✓	✓	✓		[23, 99–102]
Colony-stimulating factor and granulocyte colony-stimulating factor	✓	✓		✓		[2, 103–107]
Creatine	✓		✓	✓		[108–110]
Curcumin	✓	✓		✓		[111–113]
Inter-alpha inhibitor proteins	✓					[114, 115]
Exendin-4	✓	✓		✓	✓	[21, 116–118]

protective effects of therapeutic hypothermia on HIE involve mechanisms such as inhibiting the inflammatory cascade reactions and apoptosis; reducing the production of ROS, the concentration of free radicals, the accumulation of excitatory amino acids (glutamate and aspartate), and the permeability of BBB; maintaining or increasing antioxidant levels; and exerting endogenous neuroprotective effects [9, 52, 119]. Therapeutic hypothermia significantly reduces the incidence of cerebral palsy in HIE survivors, but the effectiveness of this treatment method is limited and cannot provide complete neuroprotection, only partially effective [120]. Clinical trials have shown that 44–53% of infants receiving hypothermia therapy will die or suffer from moderate to severe neurological dysfunction. In summary, therapeutic hypothermia can effectively reduce neurological sequelae, especially for newborns with moderate HIE, improve the survival rate of children with moderate to severe HIE, promote the development of the nervous system, and improve the long-term prognosis of newborns with HIE. However, therapeutic hypothermia cannot provide complete neuroprotection, and some patients still die from varying degrees of sequelae, indicating that using therapeutic hypothermia alone to treat HIE is not enough. It is therefore necessary to explore adjuvant therapies to therapeutic hypothermia in order to achieve better protective effects.

Drug Therapy

Clinical Trial Phase

Allopurinol After HI brain injury, xanthine oxidase, as an enzyme involved in oxidative damage, accumulates in the endothelium of cerebral capillaries, while allopurinol is an inhibitor of xanthine oxidase and can exert strong antioxidant functions [68]. In addition, allopurinol can chelate free iron and scavenge hydroxyl radicals, which helps to inhibit secondary energy failure and apoptosis of neurons and glial cells after cerebral tissue ischemia reperfusion (I/R) [24]. Allopurinol is being considered for neuroprotection and as a potential intervention for neonatal asphyxia [121, 122]. In a preliminary study of a cohort of neonates with severe HIE, it was found that intravenous injection of allopurinol in newborns resulted in reduced free radical generation and oxidative stress, improved in cerebral perfusion, and no additional toxic effects [69]. Similarly, in the case of fetal hypoxia, intravenous injection of allopurinol into the mother has been shown to reduce the S-100β (brain injury marker protein) levels and non-protein-bound iron (an oxidative stress marker) in the umbilical artery [70]. The ALBINO trial is

currently underway in European countries (effect of allopurinol on neurocognitive outcomes after hypoxic-ischemic brain injury in addition to HT therapy, NCT03162653), to assess the efficacy and safety of allopurinol immediately after birth in term infants with early signs of HIE to reduce long-term neurodevelopmental impairment [23, 123, 124]. Further research is needed to delve deeper into pathomechanism and clinical relevance.

Melatonin Melatonin is an endogenous indoleamine secreted by the pineal gland, and its function is to transmit information about the light dark cycle to the brain to regulate circadian rhythm [125]. Melatonin has antioxidant, anti-apoptotic, and anti-inflammatory properties, and can freely pass through BBB [11]. Melatonin acts as a powerful scavenger of free radicals, stimulating the expression of antioxidant enzymes and enhancing the effects of vitamin E and C [71, 72]. Melatonin inhibits NF-κB translocation and reduces pro-inflammatory cytokines [71–73]. In addition, melatonin prevents the release of cytochrome c enzyme, reduces the activation of caspase-1 and caspase-3 enzyme, prevents apoptosis by stabilizing mitochondrial function in a neonatal rat model of HIE [74, 75]. Melatonin has potential therapeutic use for HIE due to its anti-excitatory, anti-apoptotic, anti-inflammatory, and antioxidant effects in animal models, providing safe neuroprotection for adult and newborn HI brain injury [126]. The study by Pang et al. has provided evidence of the significant neuroprotective efficacy of melatonin as a standalone therapy and in combination with hypothermia in term neonatal models of hypoxia–ischemia [127]. This suggests that melatonin is a strong candidate for translation to clinical trials in infants with moderate-severe neonatal encephalopathy. Another study has shown that administering melatonin in combination with cooling at 1 h after HI brain injury can alleviate brain injury on the basis of faster recovery of dynamic electroencephalogram at 19 h after HI brain injury, improve brain energy metabolism within 48 h, and have a special protective effect on the most severely damaged area, inner capsule, and periventricular white matter in a newborn piglet asphyxia model [128]. In a phase II trial, the combination of melatonin and therapeutic hypothermia can effectively reduce oxidative stress, improve survival rate, and achieve good neurodevelopmental outcomes at 6 months of age [129]. Despite compelling evidence from preclinical studies, there is currently a lack of large-scale clinical trials to determine the efficacy and benefits of melatonin in treating HIE in children. Furthermore, thorough research is needed on optimal dosage, administration route, and timing. Larger scale randomized controlled trials involving humans are necessary.

Erythropoietin Erythropoietin (EPO) is a glycoprotein that regulates red blood cell production and can easily pass through BBB. It is currently considered a new type of neuroprotective agent, playing a crucial role in neuroprotection in various types of ischemic damage in the central and peripheral nervous systems [76, 77]. Research on EPO's neuroprotective effect focuses on HIE, demonstrating its safety and effectiveness in enhancing hypothermic brain protection. Through the EPO receptor–dependent pathway, EPO can not only activate JAK2 enzyme, leading to the translocation of NF-κB and signal transducer and activator of transcription 5 (STAT5) into the nucleus and inhibiting inflammation, but also promote the expression of anti-apoptotic factors, exerting anti-apoptotic effects and promoting cell survival [78, 79]. In 10-day-old rat pups, EPO treatment is accompanied by an increase in the expression and activity of JAK2, STAT3, and tissue inhibitor of metalloproteinase 1 (TIMP-1) protein, which are considered key mediators of EPO induced neuroprotection in HI brain injury [80]. EPO also downregulates the expression of Fas/Fas ligand (FasL) and upregulates MMP-2 levels, thereby exerting an anti-apoptotic effect in neonatal rats [81, 82]. In addition, EPO exert additional neurotrophic effects to promote neuronal differentiation [130]. Overall, EPO can alleviate acute brain injury after hypoxia and ischemia by mitigating inflammation, oxidative stress, excitotoxicity, and apoptosis. A study conducted by Lan et al. has shown that rEPO treatment can significantly reduce HI brain injury and neurobehavioral disorders in neonatal rat, which indicates that rEPO has therapeutic potential for perinatal brain injury caused by hypoxia and ischemia [131]. In preliminary randomized trials, high-dose EPO, especially combined with therapeutic hypothermia showed potential in reducing brain injury and improving prognosis in HIE infants [132]. The scientific basis and preclinical data for EPO neuroprotection are promising. Animal studies and Phase I/II clinical trials have demonstrated the favorable prognosis of EPO in newborns with HI brain injury [79, 133]. Currently, Phase II and III clinical trials are underway to assess the safety and efficacy of neuroprotective doses of EPO in the treatment of hypoxic-ischemic encephalopathy in newborns [8, 11]. However, the results of a recent Phase 3 randomized controlled trial showed that EPO did not improve brain damage in newborns with moderate or severe HIE [134]. Therefore, the effectiveness of EPO therapy for HIE requires further data to be determined.

Topiramate (TPM) Topiramate (TPM) is an anticonvulsant drug used to treat epilepsy. TPM has potential neuroprotective effects and could be a candidate drug for HI brain injury due to its ability to reduce neuronal apoptosis, inhibit microglia and astrocyte activation, decrease epileptic activity, and reduce neural excitability [83, 84]. In animal model studies such as rats and piglets, it has been proven that using TPM alone can reduce brain damage in newborns with HIE [85–88]. The randomized controlled trial about the safety

and efficacy of TPM in neonates with HIE treated with therapeutic hypothermia suggests that administration of TPM in newborns with HIE is safe [135]. At present, there is still limited research on adverse reactions and long-term effects of TPM, and more randomized controlled trials are needed to enhance the research on the use of TPM in the treatment of HIE.

Cerebrolysin Cerebrolysin is a peptide-rich preparation made by hydrolysis and purified extraction of porcine brain [136]. It has the functions of regulating inflammatory response, providing nutritional support, preventing excitotoxicity, inhibiting oxidative stress and apoptosis, and can be used to stimulate neurogenesis, support neural cell function, and promote neurological function recovery [89, 90]. Administering cerebrolysin during the therapeutic window (up to 6-month) of HIE is a potential drug treatment method being studied to promote recovery after brain injury in infants [137]. Cerebral hemolysin serves as a neuroprotective therapy for perinatal asphyxia and HIE, leading to improvement in motor and speech function deficits of infants following treatment for a period of time, with a positive therapeutic effect and no adverse safety concerns [137–139]. Clinical trials using cerebrolysin should be further expanded to obtain long-term efficacity data for the treatment of HIE.

Magnesium Sulfate Magnesium sulfate, as an NMDAR antagonist, can alleviate excitotoxicity damage and protect the brain of newborns with birth asphyxia by regulating the activity of gated NMDA channels [91]. There is evidence to suggest that administering magnesium sulfate treatment postpartum can improve the short-term neurological prognosis of term infants with moderate to severe perinatal asphyxia. However, at 6 months, no differences were found in the neurodevelopmental results [140]. There is also a study that suggests that combination therapy with magnesium sulfate and melatonin can improve the prognosis of neonates with HIE [141]. The results of another prospective pilot study indicate that in newborns with HIE, a combination therapy with EPO (300 U/kg every other day for 2 weeks), magnesium sulfate (250 mg/kg for 3 days), and therapeutic hypothermia is feasible [142]. However, the neuroprotective effect of magnesium sulfate treatment on newborns with moderate asphyxia remains to be determined.

Preclinical Research Phase

N-Acetyl-l-Cysteine (NAC) NAC, an antioxidant, rapidly replenishes glutathione and enhances neuroprotection by scavenging ROS in injured tissues, demonstrating potential for mitigating oxidative stress in HIE [143]. It plays a crucial role in protecting oligodendrocyte cells, which are particularly vulnerable to the effects of free radicals and inflammatory cytokines induced by HIE [92–94]. Studies have demonstrated NAC's neuroprotective effects both in vitro and in vivo, with the potential to inhibit apoptosis and restore motor function in animal models of HIE [92]. Furthermore, when combined with therapeutic hypothermia, NAC has been found to significantly reduce cerebral infarction volume in neonatal rats [9, 144]. Additionally, low doses of NAC combined with vitamin D have shown positive outcomes for newborns with HIE [145]. In summary, NAC can serve as a neuroprotective factor by inhibiting the oxidative stress and apoptosis of central nervous system (CNS) under HIE conditions.

Neuroprotective Gases (Hydrogen, Xenon, and Argon) In the HIE animal model, there is ongoing debate regarding the neuroprotective effects of hydrogen treatment on hypoxic-ischemic brain injury. While the majority of studies have shown positive effects, some have found no significant protection [95]. The mechanisms involved in the neuroprotective effect induced by hydrogen include reducing oxidative damage, inhibiting apoptosis, and inhibiting neuroinflammation while enhancing neurotrophic effect of microglia [95, 96]. The effectiveness of neuroprotection in neonatal rodent HIE models depends not only on the severity but also on the age of the rodents [95, 146–148]. The piglet model is commonly used for studying neonatal HIE due to its similarities with human newborns. Recently, Kovács et al. found that inhaled hydrogen does not enhance the neuroprotective effect of therapeutic hypothermia in a severe neonatal hypoxic-ischemic encephalopathy piglet model [149]. Although clinical trials of hydrogen therapy have been conducted in the adult population, there is a lack of reported trials involving neonates with HIE.

Xenon acts as an NMDAR antagonist and has neuroprotective effects, including anti-inflammatory and antioxidant effects, attenuation of excitotoxicity [97, 98]. In a newborn rat HIE model, sub-intoxicating dose xenon showed neuroprotective effects; inhalation of xenon combined with therapeutic hypothermia was superior to therapeutic hypothermia alone [150, 151]. Faulkner et al. evaluated the neuroprotective effects of combining inhaled xenon with therapeutic hypothermia in a piglet model of perinatal asphyxia. They found that xenon-augmented hypothermia reduced cerebral MRS abnormalities and cell death markers in certain brain regions compared to no treatment but did not show statistical significance when compared with hypothermia alone[152]. The utilization of xenon in the management of patients with HIE is constrained by its high expenses; however, the incorporation of a recirculation system could potentially reduce the requirement for fresh xenon and facilitate the advancement of Phase II clinical trials for xenon-enhanced hypothermia [153]. A TOBY-Xenon clinical trial conducted in the UK demonstrated that the use of xenon within the delayed

timeframe in this study is both feasible and apparently safe. However, it is unlikely to augment the neuroprotective effect of postnatal hypothermia following birth asphyxia [154]. Argon also shows potential for neuroprotection in HIE animal models, but the exact mechanism remains unclear [24, 155, 156]. Hydrogen may be a promising candidate for adjuvant therapy alongside therapeutic hypothermia in neonatal HIE due to its ability to easily penetrate the blood–brain barrier for enhanced neuroprotection and cost-effectiveness [95]. Nonetheless, further research is necessary to investigate long-term neurological outcomes as well as potential impacts on organ systems associated with these three gases.

Cannabidiol Cannabidiol, a nonpsychoactive cannabinoid found in the cannabis, exhibits neuroprotective ability in neonatal HIE animal models [99, 157, 158]. It may exert neuroprotective effects by regulating excitotoxicity, inflammation, oxidative stress, mitochondrial dysfunction, BBB integrity, and apoptosis involved in HIE [23, 100]. In newborn piglets with HIE, administering cannabidiol can prevent HI-induced protein carbonylation (a marker of increased oxidative stress), protect neurons and astrocytes, and lead to histological, functional, biochemical, and neurobehavioral improvements [99, 101]. After 7 days of HI injury in neonatal rats, it can still be observed that cannabidiol treatment can inhibit the increase in brain TNF-α concentration induced by HI injury [102]. In addition, it was found in the newborn piglet HIE model that the use of cannabinol alone or cannabinol combined with therapeutic hypothermia can significantly improve the sustained depression of brain activity and increased microglial activation caused by HI, but the use of therapeutic hypothermia alone cannot improve this situation. Administration of cannabidiol after hypoxia–ischemia in piglets demonstrates some neuroprotective effects, but the combination of cannabidiol and hypothermia exhibits an additive effect [159]. In HIE, although it is evident that cannabinol provides neuroprotection by regulating key factors such as excitotoxicity, oxidative stress, and inflammation, further analysis is needed on the exact mechanism of cannabinol's neuroprotection in neonatal HIE.

Colony-Stimulating Factor 1 (CSF1) and Granulocyte Colony-Stimulating Factor (G-CSF) Colony stimulating factor 1 (CSF1) is a key regulator of microglia growth and function. In a neonatal HIE rat model, intranasal administration of rh-CSF1 can mitigate ischemia-induced neuroinflammation and oxidative stress, decrease infarct size and brain edema, ameliorate neurobehavioral deficits by modulating the CSF1R/PLCG2 and downstream pathways, and may represent a novel therapeutic approach for improving brain injury in HIE patients [103, 104]. Granulocyte colony-stimulating factor (G-CSF), a member of the hematopoietic growth factor family, has been shown to have neuroprotective effects on neurodegenerative diseases and neonatal brain injury by inhibiting apoptosis and inflammation [2, 105–107]. G-CSF can reduce the release of pro-inflammatory mediators (TNF-α and IL-1β) to prevent the excessive activation of monocytes and lymphocytes, while simultaneously G-CSF also can activate anti-inflammatory signaling to defend neutrophils, thereby exerting anti-inflammatory and indirect anti-apoptotic effects [2]. G-CSF has neuroprotective effects by improving neuroinflammation and reducing apoptosis through the PI3K/AKT pathway, JAK/STAT3 pathway and mTOR/p70S6K pathway after neonatal HIE in rats [32, 160]. Despite these advances, there is still little research on the neuroprotective effect of G-CSF on neonatal HIE, and more research is needed to explore the neuroprotective mechanism and effect of G-CSF on neonatal brain injury.

Drug Discovery Phase

Creatine Creatine, a nitrogen-containing organic acid, naturally exists in vertebrates and can assist in providing energy to muscles and nerve cells. It not only participates in energy metabolism (synthesis of phosphocreatine) and inhibits excitotoxicity, but also has antioxidant and anti-inflammatory effects [108, 109]. Previous study has shown that creatine is a very promising neuroprotective drug that can be used to prevent or treat HIE [109, 110]. Creatine may promote neuroprotection by being absorbed into vascular endothelium and/or smooth muscle cells to improve cerebrovascular function during and after HI brain injury [109]. In addition, creatine supplementation during pregnancy can exert anti-excitotoxicity by interacting with GABAA receptor [161]. Creatine's anti-inflammatory and antioxidant properties may potentially help in treating HIE, but the specific mechanism requires further research.

Curcumin Curcumin is extracted from the rhizome of *Curcuma longa* and is the main phytochemical component of *Curcuma longa*. It has anti-inflammatory, antioxidant, antimicrobial, and anti-apoptotic effects. Clinical studies have shown that taking a dose of 8 g/day of curcumin in the human body has significant safety and good tolerance, and the small molecular weight and size of curcumin enable it to smoothly cross the BBB [111, 112]. Curcumin has neuroprotective effects, which may be the result of changes in the phosphorylated STAT3, thereby affecting inflammation and mitochondrial dysfunction after HI brain injury in neonatal mice [113]. Therefore, curcumin can be considered an effective candidate drug for neuroprotective treatment of neonatal HI brain injury. However, due to the drawbacks of poor absorption, fast metabolism, rapid systemic elimination, and poor bioavailability of curcumin [22, 162], future experiments should focus on how to improve the bioavailability and solubility of curcumin, and develop oral solutions for clinical applications.

Inter-Alpha Inhibitor Proteins (IAIPs) IAIPs are immunomodulatory proteins with anti-inflammatory properties that are believed to limit the progression of neonatal inflammation [114]. Recent study has shown that IAIPs intraperitoneal therapy can reduce neuronal cell and non-neuronal cell death, weaken glial reactivity and leukocyte invasion, reduce cerebral infarction area, and provide long-term behavioral benefits in neonatal rat HI brain injury models [115]. For newborns with HI brain injury, systemic administration of exogenous IAIPs is a promising neuroprotective strategy. However, there is currently little research on IAIPs, and further research is needed on the optimal treatment plan and the neuroprotective mechanism of IAIPs.

Exendin-4 Exendin-4 is glucagon-like peptide-1 receptor agonist with anti-inflammatory, mitochondrial protection, anti-apoptosis, antioxidant, and neurotrophic effects [21, 116, 117]. There are limited preclinical studies on the effect of exendin-4 on perinatal brain injury, but a groundbreaking study on newborn mice has shown that exendin-4 exhibits good neuroprotective effects on newborn mice with HI brain injury and enhances the brain protection of therapeutic hypothermia. This protective effect may be mediated by reducing cerebral infarction, inhibiting cell death and inflammation [118]. The exact mechanism of the neuroprotective effect of exendin-4 is still not fully understood, and further research is needed to investigate the neuroprotective effect and safety of exendin-4 on perinatal HI brain injury.

Stem Cell Therapy

Stem cell therapy may rescue and replace ischemic tissue caused by HI injury, as well as promote endogenous neural repair [24]. Stem cells have been shown to improve or modify various aspects of HI brain injury, including infarct size, apoptosis levels, axonal sprouting, neurite growth, and microglial cell activation [163]. Currently, exogenous stem cell transplantation using mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), neural stem cells (NSCs), and multilineage-differentiating stress-enduring (Muse) cells is being researched for its potential in improving functional recovery from HIE.

MSCs are the most used regenerative cells in clinical trials because they are relatively safe and easy to isolate/reproduce [164]. MSCs secrete various factors to enhance regeneration and repair of damaged cells and tissues, making them an exciting new therapeutic drug for improving HIE [165]. Umbilical cord MSCs and their derived vesicles play a neuroprotective role in hypoxic-ischemic brain injury by reducing inflammatory response and inhibiting apoptosis [166, 167]. Clinical trials using MSCs to treat HIE have been conducted, but further research is needed to optimize their efficacy and safety [168]. Umbilical cord blood (UCB), a type of medical waste obtained postpartum, shows promise for therapeutic use in neonates with HIE [15, 169]. In addition to MSCs, umbilical cord blood (UCB) also contains HSCs and EPCs, as well as regulatory T cells (Treg) and monocytes. Autologous UCB stem cells have neuroprotective effects, including anti-apoptotic and anti-inflammatory effects, reducing oxidative stress, reducing $CD4^+$ T cell infiltration, stimulating angiogenesis, and upregulating neurotrophic factors [165]. Recent studies have indicated that the intravenous infusion of human UCB $CD34^+$ cells partially improve HIE injury in neonatal mice and rats [170, 171]. Furthermore, a single-arm clinical study has demonstrated that intravenous autologous UCB is feasible and safe for treating neonatal HIE with hypothermia [172]. Additionally, regulatory T cells and monocytes mediate neuroprotective effects [2, 18, 19]. Based on the aforementioned research findings, it can be inferred that intravenous infusion of umbilical cord blood cells may represent the most optimal and promising cell therapy for treating neonatal HIE at present.

Stem cell technology has led to extensive research on NSCs transplantation for treating HIE. This approach shows promise in regenerative repair by compensating for NSCs deficiency and replacing degenerated neurons [12]. Preliminary study has shown that intranasal administration of human NSCs can improve the neurobehavioral outcomes of neonatal rats with HIE, which may be related to the regulation of NF-κB signal pathway [173]. Additionally, Muse cells are being investigated as candidate cells for regeneration due to their unique characteristics such as stress tolerance and pluripotency markers [174]. A current ongoing study aims to evaluate the safety and tolerance of allogeneic Muse cell-based product (CL2020) cells in newborns with HIE, marking the first clinical trial of CL2020 cells in newborns [175]. Overall, intravenous injection of allogeneic Muse cells is expected to become an effective regenerative therapy for treating HIE. Stem cell therapy holds the potential to replace damaged cells during and after HI injury, as well as enhance self-renewal capabilities. Clinical trials are still needed to determine the most effective cell type, optimal dosage, and administration time for achieving the best therapeutic effect of stem cell therapy for HIE, either alone or in combination with TH.

Other Therapy

Pioglitazone and gallic acid have shown therapeutic potential in research on I/R and HI brain injuries, making them promising candidates for the treatment of HIE. Pioglitazone reduces neuronal pyroptosis by activating PPAR-gamma, while gallic acid possesses anti-inflammatory and antioxidant properties[176, 177]. GPR39, a member of the G protein-coupled receptor family, reduces pro-inflammatory cytokines

and enhances anti-inflammatory cytokines post-neonatal HIE in rats. Its specific agonist TC-G 1008 may be a promising therapeutic drug for alleviating neuroinflammation in neonatal HIE [178]. With the advancement of gene editing technology, particularly Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 as a highly efficient tool for gene editing, there is an anticipation that editing genes associated with the onset and progression of HIE will emerge as a novel therapeutic approach for HIE treatment. Molecular chaperones, especially heat shock proteins (HSP), help to fold proteins correctly, prevent aggregation, and have anti-apoptotic and neuroprotective effects, providing potential for new therapeutic strategies for HIE [30, 179].

Perspectives

HIE is the brain injury developing after perinatal asphyxia, and it is an important disease related to acute neonatal death and chronic neurological damage. Most studies on HIE have been conducted on newborns and neonatal animal models. This article introduces the pathogenesis and treatment strategies of neonatal HIE. The currently reported pathologic events involved in the occurrence and development of neonatal HIE mainly include hypoxia–ischemia injury, reperfusion injury, inflammation, oxidative stress, mitochondrial dysfunction, excitotoxicity, NMDAR over-excitation, ferroptosis, ERS, and apoptosis. These multifaceted factors exhibit intricate interrelationships with each other, and existing technology makes it challenging to determine the predominant factor driving the pathology. The exploration of mechanisms still has a long way to go. We introduce the treatment of HIE from three aspects: standard therapy, drug therapy, and stem cell therapy. Therapeutic hypothermia is currently the only standard therapy used in clinical practice, although it can provide partial protection to patients, it does not provide complete neuroprotection to patients. Regarding this, it is of great significance to to explore the potential use of drug therapy or stem cell therapy as an adjuvant therapy to TH. Combination therapy may be a more effective method for treating neonatal HIE. In addition, developing new drugs and conducting more in-depth research on developed drugs are of great importance and urgency for the treatment of HIE.

Consent for Publication

Not applicable.

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Declarations

Ethics Approval and Consent to Participate Not applicable.

Competing Interests The authors declare no competing interests.

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